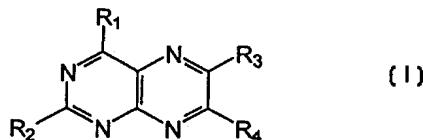




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>C07D 475/04, 475/08, A61K 31/519, A61P 35/00</b>		A1	(11) International Publication Number: <b>WO 00/39129</b>
(21) International Application Number: PCT/EP99/10320		(43) International Publication Date: 6 July 2000 (06.07.00)	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 28 December 1999 (28.12.99)			
(30) Priority Data: 60/113,989 28 December 1998 (28.12.98) US			
(71) Applicant (for all designated States except US): K.U. LEUVEN RESEARCH & DEVELOPMENT [BE/BE]; Groot Begijnhof, Benedenstraat 59, B-3000 Leuven (BE).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): WAER, Mark, Joseph, Albert [BE/BE]; Waversebaan 263, B-3001 Heverlee (BE). HERDEWIJN, Piet, André, Maurits, Maria [BE/BE]; Olivierstraat 21, B-3111 Rotselaar/Wezemael (BE). PFLEIDERER, Wolfgang, Eugen [DE/DE]; Lindauerstrasse 47, D-78464 Konstanz (DE).			
(74) Agent: HOORWEG, Petrus, Nicolaas; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).			

(54) Title: IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES



## (57) Abstract

The invention relates to a pharmaceutical composition for the treatment of autoimmune disorders or for the treatment and/or prevention of transplant-rejections and/or for the treatment of inflammatory diseases comprising as an active principle at least one pteridine derivative having general formula (I). The invention further relates to a compound having said general formula and to the use of a compound as a medicament and for the manufacture of a medicament for the treatment of autoimmune disorders, for the prevention of transplant-rejections and for the treatment of inflammatory diseases.

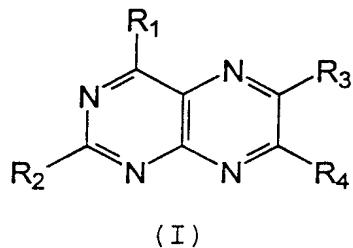
**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece	<b>ML</b>	Mali	<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>MN</b>	Mongolia	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MR</b>	Mauritania	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MW</b>	Malawi	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MX</b>	Mexico	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>NE</b>	Niger	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NL</b>	Netherlands	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NO</b>	Norway	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NZ</b>	New Zealand	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>PL</b>	Poland		
<b>CM</b>	Cameroon	<b>KR</b>	Republic of Korea	<b>PT</b>	Portugal		
<b>CN</b>	China	<b>KZ</b>	Kazakhstan	<b>RO</b>	Romania		
<b>CU</b>	Cuba	<b>LC</b>	Saint Lucia	<b>RU</b>	Russian Federation		
<b>CZ</b>	Czech Republic	<b>LI</b>	Liechtenstein	<b>SD</b>	Sudan		
<b>DE</b>	Germany	<b>LK</b>	Sri Lanka	<b>SE</b>	Sweden		
<b>DK</b>	Denmark	<b>LR</b>	Liberia	<b>SG</b>	Singapore		

## IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a pharmaceutical  
5 composition for the treatment of autoimmuno disorders or  
for the treatment and/or prevention of transplant-  
rejections and/or the treatment of inflammatory diseases  
comprising as active ingredient one or more pteridine  
derivatives having the general formula:



10

wherein:

R<sub>1</sub> and R<sub>2</sub> are independently amino, hydroxylamino,  
15 alkoxyamino, hydrazino, piperazino, N-alkylpiperazino,  
morpholino, mono- and diarylamino, (wherein the aryl group  
may be the same or different) mono- and dialkylamino  
(wherein the alkyl group may be the same or different),  
mono- and diarylalkylamino (wherein both groups may be the  
20 same or different), cycloalkylamino (such as  
cyclopropylamino, cyclobutylamino, cyclopentylamino,  
cyclohexylamino), alkoxy, mercaptoalkyl. The alkyl group  
may contain 1 to 7 carbon atoms and may be branched,  
cyclized and may be oxidized;

25 R<sub>3</sub> : unsubstituted, monosubstituted or  
disubstituted aryl group (wherein the substituent may be,  
but not limited to, halogen, alkoxy, alkyl), aryl group  
bond to the pteridine ring via a saturated or unsaturated  
alifatic spacer which may be halogenated or hydroxylated,  
30 aliphatic substituent which may contain ether function,  
alcohol function, substituted or unsubstituted amino  
functions; and

2

$R_4$  : hydrogen, alkyl, alkoxy, substituted or unsubstituted aryl.

5 The invention further relates to combined pharmaceutical preparations comprising one or more pteridine derivates and one or more known immunosuppressant, and to a group of novel pteridine derivates as such.

10 Further the invention is also related to a method for the treatment of autoimmuno disorders and/or of transplant-rejections and/or inflammatory diseases.

15 The invention further relates to a method for the preparation of the above mentioned pteridine derivatives and the the pteridine derivatives as such.

20 Several pteridine derivates are known in nature and used in the preparation of medicines, for example as described in EP-A-108 890. Other medical uses of derivates of pteridine are described in WO 95-31987 as NO-synthase inhibitors for example for the treatment of diseases caused by a high nitrogen monoxide level. Further, WO-95-32203 describes also the use of tetrahydropteridine derivates as NO-synthase inhibitors.

25 Both above-mentioned WO publications disclose also the use of specific pteridine derivates in the treatment of pathologically low blood pressure, in particular septic shock and combined with cytokines in tumor therapy and in transplant-rejection diseases.

30 Although some of these pteridine derivates are claimed as potentially active for the treatment of transplant-rejection diseases, direct evidence for their effectiveness is lacking. Overall there still is a need for specific and highly active immunosuppressive compounds, in particular immunosuppressive compounds active in the cosignal pathway.

35 A first object of the invention is to provide a pharmaceutical composition having high immunosuppressive activity. Another object of the invention is to provide a

3

combined immunosuppressive preparation which causes a superadditive effect, comprising a pteridine derivate of the invention and other known immunosuppressants.

Another further object of the invention is to  
5 provide immunosuppressive compounds, which are active in a minor dose, in order to decrease the considerable treatment costs.

Known immunosuppressive compounds are for example cyclosporine A, substituted xanthines, tacrolimus  
10 (FK 506), rapamycine (RPM), leflunomide, mofetil, adrenocortical steroids, cytotoxic drugs and antibody preparations.

The immunosuppressive effect of cyclosporine A (CyA) is already known since 1972. However, due to its  
15 nephrotoxicity and several other side effects CyA has not been able to establish itself as the optimal and final drug of choice.

Methylxanthines, for example pentoxifylline (PTX), are known having immunosuppressive effects in  
20 vitro.

Recently (Lin Y. et al, Transplantation 63 (1997) it has been found that the co-medication of an immunosuppressive compound such as cyclosporine A (CyA) or FK506 or RPM (rapamycine) with a methylxanthine  
25 derivative, in particular A802715 (7-propyl-1(5-hydroxy-5-methylhexyl)-3-methylxanthine) leads to a superadditive increase in the immunosuppressive action.

Likewise, other substituted, in particular substituted 8-phenylxanthines have been found to possess  
30 immunosuppressive effects in vitro (application EP 98.201323.7).

The present invention relates in particular to the application of a group pteridine derivates and their pharmaceutical salts, possessing unexpectedly desirable  
35 pharmaceutical properties, i.c. are highly active immunosuppressive agents, are useful in the treatment in

4

transplant rejection and/or in the treatment of inflammatory diseases.

The invention demonstrates the immunosuppressive effects of pharmaceutical compositions 5 for the treatment of autoimmuno disorders or of transplant-rejections comprising one or more pteridine derivatives of the above formula (I) or salts thereof.

The term pharmaceutically acceptable addition salt as used hereinbefore defines the non-toxic, therapeutically active addition salt forms which the compounds 10 of formula (I) may form. The compounds of formula (I) having basic properties may be converted into the corresponding therapeutically active, non-toxic acid addition salt forms by treating the free base form with a suitable 15 amount of an appropiate acid following conventional procedures. Examples of appropiate acids aaare for example, inorganic acids, for example, hydrohalic acid, e.g. hydrochloric, hydrobromic and the like acids, sulfuric acid, nitric acid, phosphoric acid and the like; or 20 organic acids, such as, for example, acetic, propanic, hydroxyacetic, 2-hydroxypropanic, 2-oxopropanic, ethanedioic, propanedioic, butanedioic, (Z)-2-butenedioic, (E)-2-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxybutanedioic, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzenesulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-25 2-hydroxybenzoic and the like acids.

The compounds of formula (I) having acidic properties may be converted in a similar manner into the 30 corresponding therapeutically active, non-toxic base addition salt forms. Examples of such base addition salt forms are, for example, the sodium, potassium, calcium salts, and also the salts with pharmaceutically acceptable amines such as, for example, ammonia, alkylamines, 35 benzathine, N-methyl-D-glucamine, hydrabamine, amino acids, e.g. arginine, lysine. The term pharmaceutically

5 acceptable addition salts also comprises the solvates which the compounds of formula (I) may form, e.g. the hydrates, alcoholates and the like.

The term stereochemically isomeric forms as used  
5 hereinbefore defines the possible different isomeric as well as conformational forms which the compounds of formula (I) may possess. Unless otherwise mentioned or indicated, the chemical designation of compounds denotes the mixture of all possible stereochemically and confor-  
10 mationally isomeric forms, said mixtures containing all diastereomers, enantiomers and/or conformers of the basic molecular structure. All stereochemically isomeric forms of the compounds of formula (I) both in pure form or in admixture with each other are intended to be embraced  
15 within the scope of the present invention.

Some compounds of the present invention may exist in different tautomeric forms and all such tautomeric forms are intended to be included within the scope of the present invention.

20 The compounds of the present invention show a broad spectrum profile as is evidenced by the results obtained in the diversity of test procedures cited hereinbefore.

An advantageous feature of the compounds of the  
25 present invention resides in their excellent oral activity; the present compounds when administered orally have been found to be practically equipotent with the same being administered subcutaneously.

A particularly important asset of most of the  
30 present compounds is their lack of sedating properties at therapeutic dose levels, a troublesome side effect associated with many antihistaminic and antiallergic compounds. The non-sedating properties of the present compounds can be demonstrated, for example, by the results obtained in studying the sleep - wakefulness cycle of the  
35 rat (Psychopharmacology, 97, 436-442, (1989)).

Another interesting feature of the present compounds relates to their fast onset of action and the favorable duration of their action.

In view of their useful properties the subject 5 compounds may be formulated into various pharmaceutical forms for administration purposes. To prepare the antiallergic compositions of this invention, an effective amount of the particular compound, in base or acid addition salt form, as the active ingredient is combined in intimate 10 admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirably in unitary dosage form suitable, preferably, for administration 15 orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid 20 preparations such as suspensions, syrups, elixirs and solutions: or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules and tablets. Because of their ease in administration, tablets and 25 capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, through other ingredients, for example to aid 30 solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, 35 suspending agents and the like may be employed. In the compositions suitable for percutaneous administration, the

carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious 5 effect on the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g. as a transdermal patch, as a spot-on or as an ointment. Acid addition salts 10 of the subject compounds due to their increased ater solubility over the corresponding base form, are obviously more suitable in the preparation of aqueous compositions.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit 15 form for ease of administration and uniformaty of dosage. Dosage unit form as used in the specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the 20 desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and 25 the like, and segregated multiples thereof.

The present invention also relates to a method of treating warm-blooded animals suffering from said 30 allergic diseases by administering to said warm-blooded animals an effective antiallergic amount of a compound of formula (I).

In general it is contemplated that an effective antiallergic amount would be from about 0.001 mg/kg to about 20 mg/kg body weight, and more preferably from about 0.01 mg/kg to about 5 mg/kg body weight.

35 The following examples are intended to illustrate and not to limit the scope of the present inventi-

only in all its aspects.

### Experimental Part

5

#### 2-amino-4-n-pentyloxy-6-styrylpteridine (1).

A mixture of 2-amino-6-chloro-4-n-pentyloxypteridine [1] (1.5 g, 5.6 mmoles) palladium acetate (63 mg, 0.28 mmoles), tri-*o*-tolylphosphane (682 mg, 2.24 mmoles), 10 cuprous iodide (53 mg, 0.28 mmoles), styrene (1.3 ml., 11.3 mmoles) and triethylamine 3.1 ml, 22 mmoles) was stirred in dry acetonitrile (50 ml.) under reflux for 90 hours. It was evaporated and the residue purified by silica gel column chromatography with CHCl<sub>3</sub>. The product 15 fraction was evaporated to give 1.37 g (72%) of an orange powder. Recrystallization from EtOAc/hexane. M.p. 127-128°C.

#### 2-Amino-6-(1,2-dibromophenethyl)-4-n-pentyloxypteridine (2).

To a solution of **1** (1.0 g, 2.94 mmoles) in chloroform (50 ml.) was added a 2 M bromine solution in chloroform (2.2 ml., 4.4 mmoles) and then the mixture stirred at room temperature for 7 hours. It was diluted with chloroform 25 (50 ml.), washed with a saturated aqueous Na<sub>2</sub>SO<sub>3</sub> solution (100 ml.) and dried over Na<sub>2</sub>SO<sub>4</sub>. It was evaporated, the residue treated with little toluene, filtered, washed with ether and dried in a vacuum desiccator to give 0.84 g (57%) yellow powder.

30

#### 2-Amino-4,7-dimethoxy-6-styrylpteridine (3).

A suspension of **2** (0.3 g, 0.6 mmoles) is abs. Methanol (10 ml.) was treated with 1 M methanolic sodium methoxide (3 ml., 3 mmoles) and then refluxed for 4 hours. It was 35 diluted with chloroform (100 ml.), washed with saturated aqueous NH<sub>4</sub>Cl solution and water and then the solution

dried over  $\text{Na}_2\text{SO}_4$ . The filtrate was evaporated and the residue purified by silica gel column chromatography in chloroform. The product fraction was evaporated to give 50 mg. (26%) of a yellow powder, M.p. 197-198°C.

5

$\text{O}^4$ -Methyl-biopterin (4).

To a solution of  $\text{N}^2$ , 1',2'-O-triacetyl-biopterin (1.0 g; 2.75 mmoles), triphenylphosphane (12.08 g, 4.13 mmoles) and methanol (0.15 ml., 3.7 mmoles) in dry dioxane (30 ml.) was added diisopropyl azodicarboxylate (0.81 g, 4.11 mmoles) and after stirring for 1.5 hours at room temperature evaporated to dryness. The residue was purified by silica gel column chromatography eluting with  $\text{EtOAc}/\text{CHCl}_3$  (1:4). The product fraction was evaporated and dried in vacuum to give 0.4 g (38%) of  $\text{N}^2$ , 1',2'-O-triacetyl- $\text{O}^4$ -methylbiopterin.

Deacetylation of the reaction product (0.28 g, 0.74 mmoles) was done by stirring in abs. Methanol (20 ml.) and triethylamine (4 ml.) for 24 hours. Evaporation to dryness, treatment of the residue with ether, filtration and drying gave 0.172 g (83%) of 4. M.p. 160-161°C (Decomp.).

25 General procedure for the synthesis of 2,4-diamino-6-arylpteridines (5, 7, 8, 9)

A suspension of 2,4,5,6-tetraaminopyrimidine dihydrochloride (2.13 g, 0.01 moles) in methanol (100 ml.) was heated to boiling and then a solution of the arylglyoxalmonoxime (phenylglyoxalmonoxime [2], p-methylphenylglyoxalmonoxime [3], p-methoxyphenylglyoxalmonoxime [4], p-chlorophenylglyoxalmonoxime [5] (0.015 moles) in methanol (20 ml.) added dropwise within 30 min. It was heated under reflux for 2 hours forming a precipitate. After 35 cooling was neutralized by conc. ammonia to pH 8 with stirring. The precipitate was collected, washed with

methanol and ether and dried in the oven at 100°C. Yield: 85-95%. The reaction product is usually chromatographically pure. Recrystallization can be achieved from DMF.

5

2,4-Diamino-7-methyl-6-phenylpteridine (6).

Analogous to the preceding procedure using  $\alpha$ -hydroximinopropiophenon. Yield: 70%.

10 General procedures for the synthesis of 4-amino-6-aryl-2- $\beta$ -hydroxyethylaminopteridines (10, 11, 12)

A suspension of 4,5,6-triamino-2- $\beta$ -hydroxyethylaminopyrimidine trihydrochloride (2.93 g, 0.01 moles) in methanol (60 ml.) was heated under reflux 15 and then a solution of the aryl-glyoxalmonoxime (0.015 moles) in methanol (15 ml.) added dropwise. After reflux for 2 hours and cooling was neutralized to pH 9 with conc. Ammonia to give a yellow precipitate. Yield: 90%.

20 2-amino-4-hydroxylamino-6-phenylpteridines (13).

A suspension of 2,5,6-triamino-4-methoxypyrimidine dihydrochloride (1 g, 4 mmoles) in methanol (40 ml.) was heated to boiling and then a solution of phenylglyoxalmonoxime (1 g, 6.6 mmoles) in methanol (10 ml.) added dropwise. A clear solution is obtained from 25 which on reflux for 2 hours a precipitate separated out. The solid was filtered off (hydrochloride salt), suspended in water (30 ml) and then neutralized to pH 8 by conc. ammonia. The precipitate was collected, washed 30 with water and ethanol and dried at 100°C to give a yellow powder. Yield: 0.84 g (82%).

2,4-diamino-6-bromomethylpteridine [6].

A suspension of 2,4,5,6-tetraaminopyrimidine 35 trihydrobromide (3.0 g, 0.01 moles) in methanol (60 ml) was heated to reflux and then a solution of  $\beta$ -

11

bromopyruivaldoxime (0.015 moles) in methanol (30 ml) added dropwise within 10 min. The resulting yellow solution was refluxed for 30 min., then cooled to room temperature and neutralized by conc. ammonia to pH 8.

5 The yellow precipitate was collected, washed with little methanol and ether and dried in a vacuum desiccator. Yield: 88%.

10 General procedure for 2,4-diamino-6-alkoxymethyl-(17,18) and -6-aminomethylpteridines (19,20).

To a mixture of dimethylacetamide (DMA) (30 ml) and the appropriate alcohol ( $\beta$ -methoxyethanol, n-decanol) (5 ml) was added sodium hydride (1 g, 80%) and after stirring for 1 hour 2,4-diamino-6-bromomethylpteridine (1 g) 15 added. Stirring was continued at room temperature for 6 hours, then diluted with  $H_2O$  (100 ml) and kept in the icebox for 2 days. The precipitate was collected and recrystallized from EtOH/ conc.  $NH_3$  (16:1). Yield: 50%.

20 An analogous reaction takes place with amines (dimethylamine in ethanol, benzylamine) (0.04 mmoles) in DMA (20 ml) and 2,4-diamino-6-bromomethylpteridine (2.55 g, 0.01 moles). Yield: 50-60%.

25 General procedures for the synthesis of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidines.

A solution of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidine [7] (5.0 g, 16.6 mmoles) in DMF (50ml) and the appropriate amine (dimethylamine in ethanol (50%), diethylamine, di-n-propylamine, 30 dibenzylamine, morpholine, piperidine, pyrrolidine, piperazine, N-methylpiperazine) (10.0 g) was heated in an oilbath to 70°C for 5 hours. Then water (50 ml) was added, cooled and the yellow precipitate collected, washed with water and dried. Recrystallization from EtOH 35 or DMF/water. Yield: 55-90%.

12

General procedure for the synthesis of 2,5,6-triamino-4-dialkylaminopyrimidines.

A suspension of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidine (3.28 g, 10 mmoles) in methanol (70 ml) and conc. ammonia (10 ml) was reduced in a shaking apparatus under H<sub>2</sub> atmosphere in presence of Raney nickel catalyst (3.5 g) for 2 days. The catalyst was filtered off under argon atmosphere and then the filtrate evaporated in vacuo to dryness. The residue was treated with ether to remove the p-chloroaniline, filtered and then the solid stirred in methanolic HCl (10%, 50 ml) overnight. The dihydrochloride salt was collected and dried in a vacuum desiccator over KOH. Yield: 85-90%.

15 General procedure for the synthesis of 2-amino-4-dialkylamino-6-arylpteridines (14-16, 21-49)

To a boiling solution of the 2,5,6-triamino-4-dialkylaminopyrimidine dihydrochloride salt (5 mmoles) in MeOH (20 ml) was added a solution of the arylglyoxalmonoxime (7.5 mmoles) in MeOH (10 ml) dropwise and then the mixture heated under reflux for 3 hours. After cooling the suspension or solution was made alkaline by conc. ammonia to pH 9 and the resulting precipitate filtered off, washed with water and dried. Recrystallization was done from EtOH and DMF/H<sub>2</sub>O, respectively, to give a yellow solid. Yield: 50-85%.

Further compounds 50-66 were prepared according to the above described syntheses and tested.

13

## References

[1] D. Mohr, Z. Kazimierczuk, W. Pfeiderer, *Helv. Chim. Acta* **1992**, 75, 2317.

[2] L. Classen, O. Manasse, *Ber. Dtsch. Chem. Ges.*, **1887**, 20, 2194.

[3] I. Lalezari, *J. Org. Chem.*, **1968**, 33, 4281.

[4] J.W.G. DeMeester, H.C. van der Plas, *J. Heterocycl. Chem.*, **1987**, 24, 441.

[5] W. Borsche, *Ber. Dtsch. Chem. Ges.*, **1929**, 62, 1360.

10 [6] J.R. Piper, J.A. Montgomery, *J. Org. Chem.*, **1977**, 42, 208.

[7] W.R. Boon, T. Leigh, *Brit.*, 342, Aug. 13, 1952;  
*C.A.*, **1953**,

47

**Materials and methods**

Various models may be used for testing an immunosuppressive effect. In vivo, for example, different transplantation models are available. They are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response. In vitro, there exist also various models. The most used are lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation:

- coculture of lymphocytes of different species (MLR = mixed lymphocyte reaction): lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically.
- 20 - CD3 assay: here there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against the CD3 molecule located on the lymphocyte membrane. This molecule has a costimulatory function. The interaction anti-CD3 (= OKT3)-CD3 results in T-cell activation which proceeds via the  $\text{Ca}^{2+}$ /calmodulin/calcineurin system and can be inhibited by CyA.
- 25 - CD28 assay: here specific activation of the T-lymphocyte goes also via an exogenously added antibody against the CD28 molecule. This molecule is also located on the lymphocyte membrane, and delivers strong costimulatory signals. This activation is  $\text{Ca}^{2+}$ -independent and thus cannot be inhibited by CyA.

35 Reagents

All derivatives were dissolved in 0.5 ml DMSO and

15

further diluted in culture medium before use in in vitro experiments. The culture medium consisted of RPMI-1640 + 10% FCS.

5 Mixed Lymphocyte Reaction

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or EBV-transformed 10 human B cells [RPMI1788 (ATCC name CCL156)] which strongly express B7-1 and B7-2 were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37EC, 1  $\mu$ Ci [3H]-thymidine was added to each cup. After a further 16 15 hours incubation, cells were harvested and counted in a  $\beta$ -counter.

The percent suppression of proliferation by drugs was counted using the formula

$$\text{Percent inhibition} = \frac{(\text{cpm} + \text{drugs}) - \text{cpm Cult. Med}}{(\text{cpm} - \text{drugs}) - \text{cpm Cult. Med.}} \times 100$$

20

T cell purification

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The 25 resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Collect Human T (Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with this procedure. After depletion, the resulting T cell 30 preparation was highly purified explaining these cells could not be activated by PHA or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

35 Measurements of T cell proliferations induced by anti-CD3

16  
mAb + PMA or anti-CD28 mAb + PMA

Highly purified T cells ( $10^6$ /ml) were stimulated by immobilized anti-CD3 or anti-CD28 mAb in the presence of 5 PMA. Anti-CD3 mAb (CLB-CD3; CLB, Amsterdam, The Netherlands) were fixed on the 96-microwell plates by incubating the wells with 50  $\mu$ l of mAb solution (1/800 dilution in culture medium). Anti-CD28 mAb (CLB-CD28; CLB, Amsterdam, The Netherlands) 50  $\mu$ l (1/650 dilution in 10 culture medium) was added directly to the wells. Further, 20  $\mu$ l PMA (Sigma, St. Louis, MO, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20  $\mu$ l of immunosuppressants were added by serial dilution in triplicate wells. Finally 100  $\mu$ l of the T cell suspension 15 (10 $^6$ /ml) was added. After 48-hour incubation at 37°C in 5% CO<sub>2</sub> 20  $\mu$ l BrdU (100  $\mu$ M solution) (Cell Proliferation Elisa, Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation the T cell proliferation was measured using a colorimetric 20 immunoassay for qualification of cell proliferation based on measurements of the incorporation of BrdU during DNA synthesis. The optical density (OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). The percent suppression of 25 proliferation by drugs was counted using the formula:

$$\text{Percent inhibition} = \frac{(\text{OD} + \text{drugs}) - (\text{OD Cult. Med.})}{(\text{OD} - \text{drugs}) - (\text{OD Cult. Med.})} \times 100$$

30

In vitro immunosuppressive effect of Pteridine Derivatives as measured with the MLR and with tests involving polyclonal T cell proliferation induced by 35 anti-CD3 mAb + PMA or anti-CD28 mAb + PMA (table II)

17

- Table II shows the IC50 values of the various substances in the MLR. The IC50 value represents the lowest concentration of the substances that resulted in a 50% suppression of the MLR. These concentrations are divided into four subranges i.e.

5

0 stands for concentrations of at least 151  $\mu$ M,

10

+ stands for concentrations 16-150  $\mu$ M,

++ stands for concentrations 1-15  $\mu$ M,

+++ stands for concentrations lower than 1  $\mu$ M,

15

- Column III shows the IC50 value of the various substances for the anti-CD3 mAb + PMA pathway and row IV the IC50 values of the various substances for the anti-CD28 mAb + PMA pathway.

15 - As a comparison the values of other immunosuppressants: CsA, FK506, Rapamycin, Leflunomide and Mycophenolic acid methotrexate (MTX) and 5-Fluoro-uracil (5-FU) in table III are given as well.

20

First, most of the pteridine classes (I) according to the invention contain compounds with a clear suppressive effect in the MLR (mixed lymphocyte reaction). The MLR is considered as an in vitro analogue of the transplant rejection as it is based on the recognition of allogeneic MHC (major histocompatibility antigens) on the stimulator leucocytes, by responding lymphocytes. Various established immunosuppressive drugs are known to suppress the MLR, and were also shown in this description.

25

From these data it can be deduced that the pteridine derivatives are effective in clinical situations where other immunosuppressants are active as well.

These include the prevention and/or treatment of

18

organ transplant rejection, the prevention and/or treatment of both rejection and the occurrence of graft-versus-host-disease after BM transplantation; the prevention and/or treatment of autoimmune diseases including diabetes mellitus, multiple sclerosis, glomerulonephritis, rheumatoid arthritis, psoriasis systemic diseases such as vasculitis; scleroderma, polymyositis, autoimmune endocrine disorders (thyroiditis), ocular diseases (uveitis), inflammatory bowel diseases (Crohn's disease, colitis ulcerosa), autoimmune liver diseases (autoimmune hepatitis, primary biliary cirrhosis) autoimmune pneumonitis and auto-immune carditis.

Whereas cyclosporine A and FK506 are only active in the anti-CD3 + PMA test, the pteridine derivatives according to the invention were active, not only in the anti-CD3 + PMA but also in the anti-CD28 + PMA test. It has been shown that the latter is Ca-calmodulin resistant, and resistant to CsA and FK506. The anti-CD28 + PMA pathway has also been called the cosignal pathway and is important to induce energy and even tolerance in T cells. Moreover, representative compounds have been found to be active in an whole blood assay.

Under the term "organ" in the description is understood all organs or parts of organs (even several) in mammals, in particular humans, for example kidney, heart, skin, liver, muscle, cornea, bone, bone marrow, lung, pancreas, intestine or stomach.

After organ transplantation, rejection of the transplanted organ by the recipient occurs (host-versus-graft reaction). After bone marrow transplantation, also rejection of the host by the grafted cell may occur (graft-versus-host reaction). Rejection reactions mean all reactions of the recipient body or of the

19

transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or adversely affect the functional 5 ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions.

Auto-immune disorders include, *inter alia*, systemic lupus erythematosus, rheumatoid arthritis, 10 psoriasis, pemphigus, atopic dermatitis, myositis, multiple sclerosis, nephrotic syndrome (in particular glomerulonephritis), ulcerative colitis or juvenile diabetes.

An additive or synergetic effect of pteridine 15 derivatives and other immunosuppressants may be anticipated. This may be especially, although not exclusively the case for combinations with CyA or FK 506 as the latter are not suppressive in the aCD28 pathway of 20 T cell activation (table III) whereas most Pteridine derivatives are.

The invention further relates to the use of cyclosporin A or FK506 or Rapamycine and at least one pteridine derivative according to the invention for the production of a pharmaceutical for inhibiting the 25 replication of viruses such as picorna-, toga-, bunya-, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, varicella-zoster-virus or human immunodeficiency virus (HIV); or for treating of cancer 30 such as lung cancers, leukaemia, ovarian cancers, sarcoma, Kaposi's sarcoma, meningioma, colon cancers, lymph node tumors, glioblastoma multiforme, prostate cancers or skin carcinoses.

The invention further relates to the use of

20

cyclosporin A or FK506 or rapamycin and at least one pteridine derivative of the general formula (I) for the production of a pharmaceutical for the treatment of human after organ transplantation or of (auto)immune disorders.

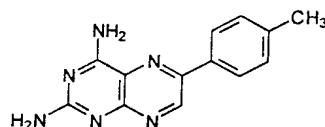
5        Hence, the advantage to associate pteridine with other immunosuppressants may be that, first, the therapeutic spectrum of action of the individual components is quantitatively and qualitatively broadened. Secondly that it allows, by means of a dose reduction  
10 without reduced efficacy but with increased safety, that the treatment of immune disorders which were hitherto no indication for immunosuppressive therapy as a result of side effects may be considered. At the same time, the therapy costs can be decreased to an appreciable extent.

15        As a comparison, known pteridine derivatives are submitted to the same test conditions as the pteridine derivatives of the invention. These compounds and the results thereof are given in table IV and show no particular immunosuppressive activity.

20        As been stated above the invention also relates to new pteridine derivatives as such, in particular the compounds 1,2,3,6, 14-16 and 21-66 and their pharmaceutically acceptable salts.

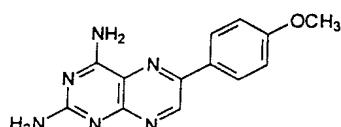
Compound n°	MLR	aCD3	aCD28
1 2-amino-4-pentoxy-6-styrylpteridine	15	110	80
2 2-amino-4-n-pentoxy-6-(1,2-dibromo-2-phenylethyl)pteridine	12	4	12
3 2-amino-4-methoxy-6-styryl-7-methoxyppteridine	25	160	100
4 2-amino-4-methoxy-6-(1,2-dihydroxypropyl)pteridine	>200	140	110
5 2,4-diamino-6-phenyl pteridine	>200	>200	>200
6 2,4-diamino-6-phenyl-7-methylpteridine	>200	>200	>200

## 7 2,4-diamino-6-(4-tolyl)pteridine



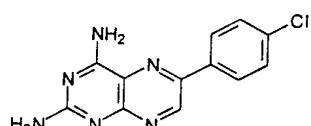
&gt;200 110 25

## 8 2,4-diamino-6-(4-methoxyphenyl)pteridine



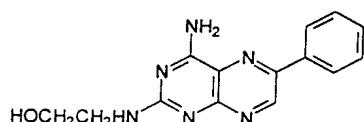
&gt;150 125 22

## 9 2,4-diamino-6-(4-chlorophenyl)pteridine



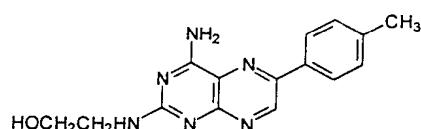
125 110 110

## 10 2-hydroxyethylamino-4-amino-6-phenylpteridine



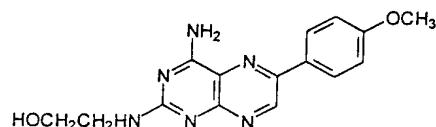
125 125 125

## 11 2-hydroxyethylamino-4-amino-6-(4-tolyl)pteridine



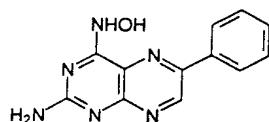
&gt;200 200 160

## 12 2-hydroxyethylamino-4-amino-6-(4-methoxyphenyl)pteridine



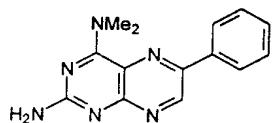
&gt;200 &gt;200 160

## 13 2-amino-4-hydroxylamino-6-phenylpteridine



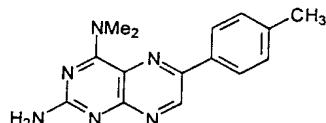
140 &gt;200 &gt;200

## 14 2-amino-4-dimethylamino-6-phenylpteridine



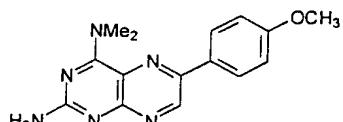
5.0 15 15

## 15 2-amino-4-dimethylamino-6-(4-tolyl)pteridine



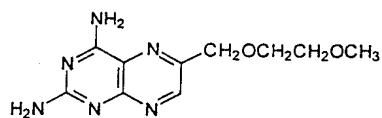
3.6 4.5 3.9

## 16 2-amino-4-dimethylamino-6-(4-methoxyphenyl)pteridine



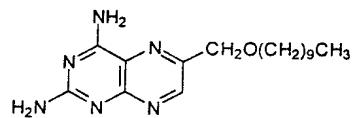
12 7.5 7.5

## 17 2,4-diamino-6-methoxyethoxymethyl pteridine



&gt;200 180 18

## 18 2,4-diamino-6-decyloxyethyl pteridine

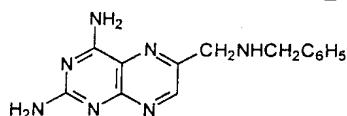


&gt;200 152 100

## 19 2,4-diamino-6-benzylaminomethyl pteridine

50 123 118

24

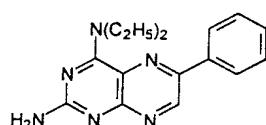


20 2,4-diamino-6-dimethyl aminomethyl pteridine



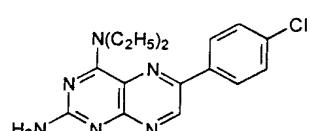
&gt;200 170 100

21 2-amino-4-diethylamino-6-phenylpteridine



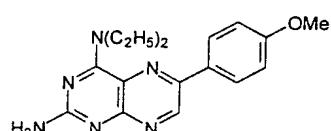
15 80 20

22 2-amino-4-diethylamino-6-(4-chlorophenyl) pteridine



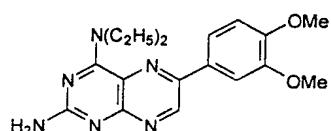
17 50 20

23 2-amino-4-diethylamino-6-(4-methoxyphenyl) pteridine



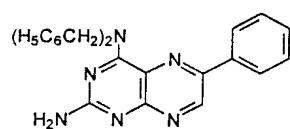
12 20 20

24 2-amino-4-diethylamino-6-(3,4-dimethoxyphenyl) pteridine



0.08 0.5 0.1

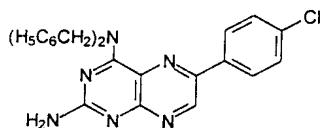
25 2-amino-4-dibenzylamino-6-phenyl pteridine



75 25 25

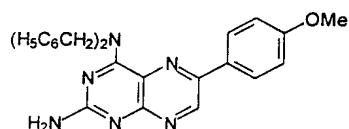
25

## 26 2-amino-dibenzylamino-6-(4-chlorophenyl) pteridine



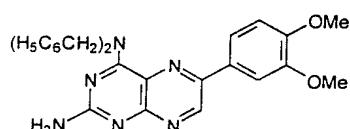
100 75 20

## 27 2-amino-4-dibenzylamino-6-(4-methoxyphenyl) pteridine



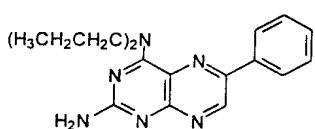
50 20 15

## 28 2-amino-4-dibenzylamino-6-(3,4-dimethoxyphenyl) pteridine



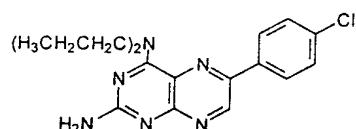
3.5 4.3 3.2

## 29 2-amino-4-dipropylamino-6-phenylpteridine



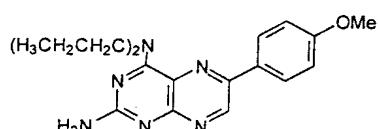
15 25

## 30 2-amino-4-dipropylamino-6-(4-chlorophenyl)pteridine



13 9.7 6.7

## 31 2-amino-4-dipropylamino-6-(4-methoxyphenyl)pteridine



8.5 5.7 2.8

## 32 2-amino-4-dipropylamino-6-(3,4-dimethoxyphenyl)pteridine



## 33 2-amino-4-morpholino-6-phenylpteridine



## 34 2-amino-4-morpholino-6-(4-chlorophenyl)pteridine



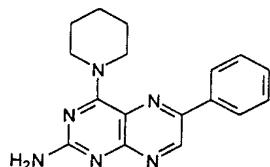
## 35 2-amino-4-morpholino-6-(4-methoxyphenyl)pteridine



## 36 2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)pteridine

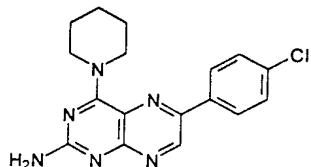


## 37 2-amino-4-piperidino-6-phenylpteridine



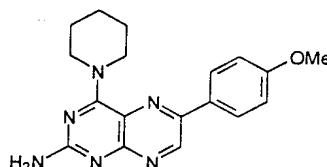
15 80 20

## 38 2-amino-4-piperidino-6-(4-chlorophenyl)pteridine



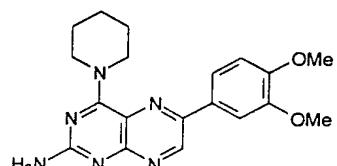
15 70 25

## 39 2-amino-4-piperidino-6-(4-methoxyphenyl)pteridine



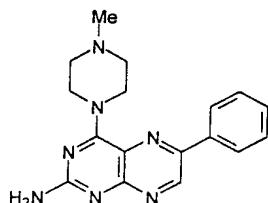
4.6 14 12

## 40 2-amino-4-piperidino-6-(3,4-dimethoxyphenyl)pteridine



0.4 0.5 0.4

## 41 2-amino-4-N-methylpiperazino-6-phenylpteridine



15 40 25

## 42 2-amino-4-N-methylpiperazino-6-(4-chlorophenyl)pteridine



## 43 2-amino-4-N-methylpiperazino-6-(4-methoxyphenyl)pteridine



## 44 2-amino-4-methylpiperazino-6-(3,4-dimethoxyphenyl)pteridine



## 45 2-amino-4-cyclopentylamino-6-(4-methoxyphenyl)pteridine



## 46 2-amino-4-piperazino-6-phenylpteridine



## 47 2-amino-4-piperazino-6-(4-chlorophenyl)pteridine



## 48 2-amino-4-piperazino-6-(4-methoxyphenyl)pteridine



## 49 2-amino-4-piperazino-6-(3,4-dimethoxyphenyl)pteridine



## 50 2-amino-4-dibenzylamino-6-(3,4,5-trimethoxyphenyl)pteridine



## 51 2-amino-4-morpholino-6-(3,4,5-trimethoxyphenyl)pteridine



## 52 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)pteridine



## 53 2-amino-4-adamantyl-6-naftypteridine



## 54 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)pteridine



## 55 2-amino-4-adamantyl-6-naftypteridine




---

## 56 2-amino-4-morpholino-6-(3,4-formylidene-3,4-dihydroxyphenyl)pteridine



## 57 2-amino-4-dimethylamino-6-(3,4-formylidene-3,4-dihydroxyphenyl)pteridine



## 58 2-amino-4-cyclopentylamino-6-(3,4-dimethoxyphenyl)pteridine



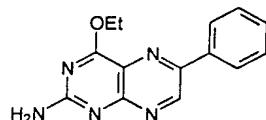
## 59 2-amino-4-dimethylamino-6-(3,4-dimethoxyphenyl)pteridine



## 60 2-amino-4-dimethylamino-6-methylpteridine



## 61 2-amino-4-ethoxy-6-phenylpteridine



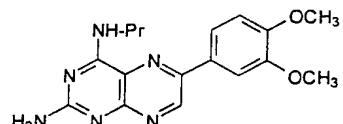
++      ++      ++

## 62 2-amino-4-propylamino-6-phenylpteridine



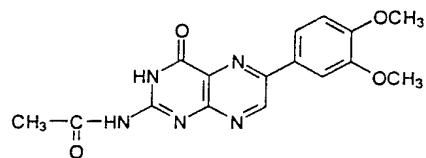
++      +      +

## 63 2-amino-4-propylamino-6-(3,4-dimethoxyphenyl)pteridine



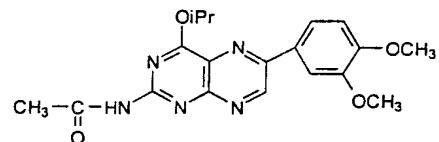
+++      ND      ND

## 64 2-acetamido-4-hydroxy-6-(3,4-dimethoxyphenyl)pteridine



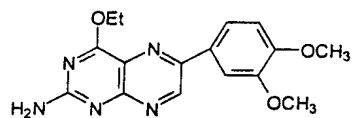
+      0      +

## 65 2-acetamido-4-i-propoxy-6-(3,4-dimethoxyphenyl)pteridine



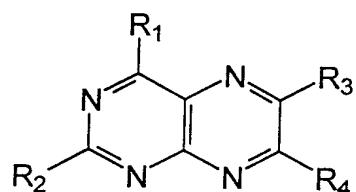
++      +      +

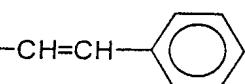
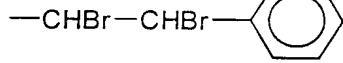
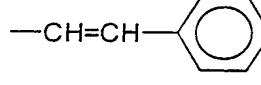
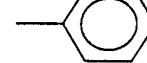
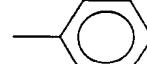
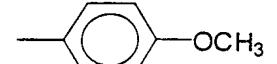
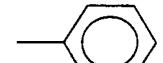
66 2-amino-4-ethoxy-6-(3,4-dimethoxyphenyl)pteridine



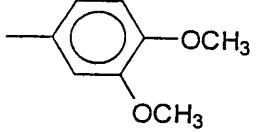
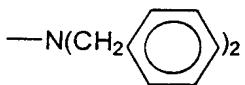
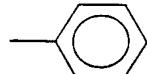
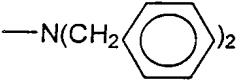
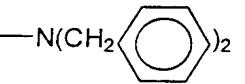
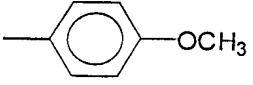
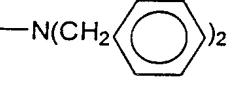
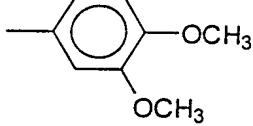
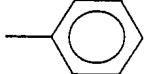
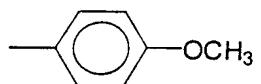
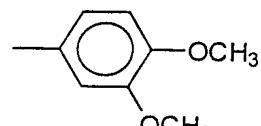
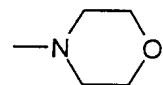
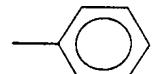
ND        +++        +++

Table I



n°	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
1	OC <sub>5</sub> H <sub>11</sub>	NH <sub>2</sub>	—CH=CH— 	H
2	OC <sub>5</sub> H <sub>11</sub>	NH <sub>2</sub>	—CHBr—CHBr— 	H
3	OC <sub>5</sub> H <sub>11</sub>	NH <sub>2</sub>	—CH=CH— 	OCH <sub>3</sub>
4	OCH <sub>3</sub>	NH <sub>2</sub>	—CHOH—CHOH—CH <sub>3</sub>	H
5	NH <sub>2</sub>	NH <sub>2</sub>	— 	H
6	NMe <sub>2</sub>	NH <sub>2</sub>	— 	CH <sub>3</sub>
7	NH <sub>2</sub>	NH <sub>2</sub>	—  —CH <sub>3</sub>	H
8	NH <sub>2</sub>	NH <sub>2</sub>	—  —OCH <sub>3</sub>	H
9	NH <sub>2</sub>	NH <sub>2</sub>	—  —Cl	H
10	NH <sub>2</sub>	NHCH <sub>2</sub> CH <sub>2</sub> OH	— 	H

			35	
11	NH <sub>2</sub>	NHCH <sub>2</sub> CH <sub>2</sub> OH		H
12	NH <sub>2</sub>	NH-CH <sub>2</sub> CH <sub>2</sub> OH		H
13	NHOH	NH <sub>2</sub>		H
14	NMe <sub>2</sub>	NH <sub>2</sub>		H
15	NMe <sub>2</sub>	NH <sub>2</sub>		H
16	NMe <sub>2</sub>	NH <sub>2</sub>		H
17	NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>2</sub> OCH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	H
18	NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>2</sub> O(CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub>	H
19	NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>2</sub> NHCH <sub>2</sub> -	H
20	NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	H
21	NEt <sub>2</sub>	NH <sub>2</sub>		H
22	NEt <sub>2</sub>	NH <sub>2</sub>		H
23	NEt <sub>2</sub>	NH <sub>2</sub>		H

		36		
24	NEt <sub>2</sub>	NH <sub>2</sub>		H
25	—N(CH <sub>2</sub>  ) <sub>2</sub>	NH <sub>2</sub>		H
26	—N(CH <sub>2</sub>  ) <sub>2</sub>	NH <sub>2</sub>		H
27	—N(CH <sub>2</sub>  ) <sub>2</sub>	NH <sub>2</sub>		H
28	—N(CH <sub>2</sub>  ) <sub>2</sub>	NH <sub>2</sub>		H
29	—N(CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NH <sub>2</sub>		H
30	—N(CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NH <sub>2</sub>		H
31	—N(CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NH <sub>2</sub>		H
32	—N(CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	NH <sub>2</sub>		H
33		NH <sub>2</sub>		H

		37		
34		NH <sub>2</sub>		H
35		NH <sub>2</sub>		H
36		NH <sub>2</sub>		H
37		NH <sub>2</sub>		H
38		NH <sub>2</sub>		H
39		NH <sub>2</sub>		H
40		NH <sub>2</sub>		H
41		NH <sub>2</sub>		H
42		NH <sub>2</sub>		H
43		NH <sub>2</sub>		H

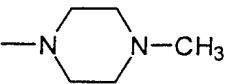
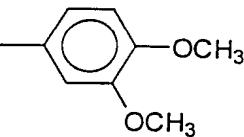
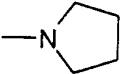
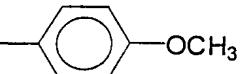
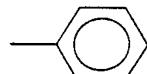
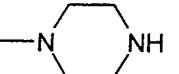
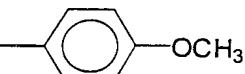
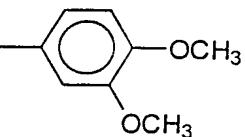
		38		
44		NH <sub>2</sub>		H
45		NH <sub>2</sub>		H
46		NH <sub>2</sub>		H
47		NH <sub>2</sub>		H
48		NH <sub>2</sub>		H
49		NH <sub>2</sub>		H

Table II

Compound n°	IC <sub>50</sub> in µM of pteridine derivative		
	MLR	ACD3	aCD28
1	15	110	80
2	12	4	12
3	25	160	100
4	>200	140	110
5	>200	>200	>200
6	>200	>200	>200
7	>200	110	25
8	150	125	22
9	125	110	110
10	125	125	125
11	>200	200	160
12	>200	>200	160
13	140	>200	>200
14	5.0	15	15
15	3.6	4.5	3.9
16	12	7.5	7.5
17	>200	180	18
18	>200	152	100
19	50	123	118
20	>200	170	100
21	15	80	20
22	17	50	20
23	12	20	20
24	0.08	0.5	0.1
25	75	25	25

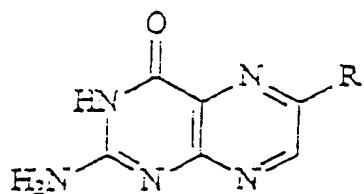
		40	
26	100	75	20
27	50	20	15
28	3.5	4.3	3.2
29	15	25	25
30	13	9.7	6.7
31	8.5	5.7	2.8
32	2.2	0.8	0.7
33	15	100	20
34	12	20	20
35	15	125	20
36	0.1	0.4	0.3
37	15	80	20
38	15	70	25
39	4.6	14	12
40	0.4	0.5	0.4
41	15	40	25
42	4.8	11	4.2
43	12.3	11.7	4.2
44	2	7.3	6.5
45	22	17	3.7
46	12	11.7	5
47	2	20	15
48	10.5	14	4.5
49	2.8	14.8	4.5

Table III

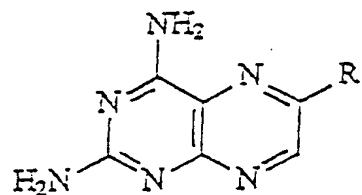
I.S.	IC50		
	Immunosuppressant		
	MLR	aCD3	aCD28
CyA	20 nM	50 nM	N.S.
FK506	1 nM	1 nM	N.S.
Rapamycin	1 nM	1 nM	1 nM
Leflunomide	25 $\mu$ M	15 $\mu$ M	20 $\mu$ M
Mofetil	<0.5 $\mu$ M	50 nM	50 nM
MTX	10 $\mu$ M	>200 $\mu$ M	> 200 $\mu$ M
5-FU		50 $\mu$ M	17 $\mu$ M

N.S. = not suppressive even not in the highest  
Concentration

Table IV



R	MLR	aCD3	aCD28
CH <sub>2</sub> OOOCCH <sub>3</sub>	0	0	0
CH <sub>2</sub> OC <sub>2</sub> H <sub>5</sub>	0	0	0
CH <sub>2</sub> NHCH <sub>3</sub>	0	0	0
CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	0	0	0
CH <sub>2</sub> S CH <sub>3</sub>	0	0	0

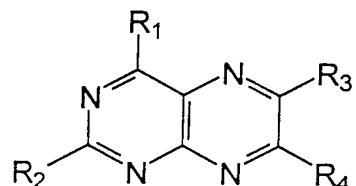


R	MLR	aCD3	aCD28
CH <sub>2</sub> S CH <sub>3</sub>	0	0	0
CH <sub>2</sub> O CH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub>	0	0	0
CH <sub>2</sub> O CH <sub>3</sub>	0	0	0
CH <sub>2</sub> O CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	0	0	0
CH <sub>2</sub> NH CO CH (CH <sub>3</sub> ) <sub>2</sub>	0	0	0

## CLAIMS

1. Pharmaceutical composition for the treatment of autoimmuno disorders or for the treatment and/or prevention of transplant-rejections and/or for the treatment of inflammatory diseases comprising as an active principle at least one pteridine derivative having the general formula:

10



(I)

15 wherein:

R<sub>1</sub> and R<sub>2</sub> are independently amino, hydroxylamino, (mono- or di)C<sub>1-7</sub>alkylamino, (mono- or di)C<sub>1-7</sub>alkyloxyamino, (mono- or di)arylamino, (mono- or di)C<sub>3-10</sub>cycloalkylamino, (mono- or di)hydroxyC<sub>1-7</sub>alkylamino, (mono- or di)C<sub>1-4</sub>alkylarylamino, mercaptoC<sub>1-7</sub>alkyl, C<sub>1-7</sub>alkyloxy or a saturated or unsaturated heterocyclic compound containing at least one nitrogen and optionally substituted by one or more C<sub>1-4</sub>alkyl, hydroxyC<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyloxy, halo, hydroxy, hydroxycarbonyl, C<sub>1-4</sub>alkyloxycarbonyl,

25 R<sub>3</sub> is an unsubstituted, monosubstituted or disubstituted aryl group (wherein the substituent may be, but not limited to, halogen, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl), aryl group bond to the pteridine ring via a saturated or unsaturated aliphatic spacer which may be halogenated or 30 hydroxylated, aliphatic substituent which may contain ether function, alcohol function, substituted or unsubstituted amino functions or C<sub>1-4</sub>alkyloxy,

**44**

R<sub>4</sub> is hydrogen, alkyl, alkoxy, substituted or unsubstituted aryl

or a pharmaceutical acceptable addition salt or a stereochemical isomeric form thereof.

5 2. Pharmaceutical composition according to claim 1, wherein R<sub>1</sub> is di(C<sub>1-4</sub>alkyl)amino, morpholinyl, piperidinyl, piperazinyl, C<sub>1-4</sub>alkylpiperazinyl, pyrroldinyl or benzylamine.

10 3. Pharmaceutical composition according to claim 1 or 2, wherein R<sub>2</sub> is ammonium, hydroxyammonium, (mono- or di)hydroxylC<sub>1-7</sub>alkylammonium.

15 4. Pharmaceutical composition according to claim 1, 2 or 3, wherein R<sub>3</sub> is benzyl, phenyl, styryl, phenyl-(C<sub>1-4</sub>alkyloxy, phenyl(C<sub>1-4</sub>alkyl) optionally substituted by one or more C<sub>1-4</sub>alkyl or halo.

5. Pharmaceutical composition according to claims 1-4, wherein R<sub>4</sub> is hydrogen or C<sub>1-4</sub>alkyl.

20 6. Pharmaceutical composition according to claims 1-5, wherein the pteridine derivative is a compound chosen from the group comprising:

1 2-amino-4-pentoxy-6-styrylpteridine

2 2-amino-4-n-pentoxy-6-(1,2-dibromo-2-phenyl ethyl)pteridine

3 2-amino-4-methoxy-6-styryl-7-methoxypteridine

25 4 2-amino-4-methoxy-6-(1,2-dihydroxypropyl)pteridine

5 2,4-diamino-6-phenyl pteridine

6 2,4-diamino-6-phenyl-7-methylpteridine

7 2,4-diamino-6-(4-tolyl)pteridine

8 2,4-diamino-6-(4-methoxyphenyl)pteridine

30 9 2,4-diamino-6-(4-chlorophenyl)pteridine

10 2-hydroxyethylamino-4-amino-6-phenylpteridine

11 2-hydroxyethylamino-4-amino-6-(4-tolyl)pteridine

12 2-hydroxyethylamino-4-amino-6-(4-methoxyphenyl)pteridine

35 13 2-amino-4-hydroxylamino-6-phenylpteridine

14 2-amino-4-dimethylamino-6-phenylpteridine

## 45

15 2-amino-4-dimethylamino-6-(4-tolyl)pteridine  
16 2-amino-4-dimethylamino-6-(4-methoxyphenyl)pteridine  
17 2,4-diamino-6-methoxyethoxymethyl pteridine  
18 2,4-diamino-6-decyclooxymethyl pteridine  
5 19 2,4-diamino-6-benzylaminomethyl pteridine  
20 2,4-diamino-6-dimethyl aminomethyl pteridine  
21 2-amino-4-diethylamino-6-phenylpteridine  
22 2-amino-4-diethylamino-6-(4-chlorophenyl) pteridine  
23 2-amino-4-diethylamino-6-(4-methoxyphenyl) pteridine  
10 24 2-amino-4-diethylamino-6-(3,4-dimethoxyphenyl)-  
pteridine  
25 2-amino-4-dibenzylamino-6-phenyl pteridine  
26 2-amino-dibenzylamino-6-(4-chlorophenyl) pteridine  
27 2-amino-4-dibenzylamino-6-(4-methoxyphenyl)pteridine  
15 28 2-amino-4-dibenzylamino-6-(3,4-dimethoxyphenyl)-  
pteridine  
29 2-amino-4-dipropylamino-6-phenylpteridine  
30 2-amino-4-dipropylamino-6-(4-chlorophenyl)pteridine  
31 2-amino-4-dipropylamino-6-(4-methoxyphenyl)pteridine  
20 32 2-amino-4-dipropylamino-6-(3,4-dimethoxyphenyl)-  
pteridine  
33 2-amino-4-morpholino-6-phenylpteridine  
34 2-amino-4-morpholino-6-(4-chlorophenyl)pteridine  
35 2-amino-4-morpholino-6-(4-methoxyphenyl)pteridine  
25 36 2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)pteridine  
37 2-amino-4-piperidino-6-phenylpteridine  
38 2-amino-4-piperidino-6-(4-chlorophenyl)pteridine  
39 2-amino-4-piperidino-6-(4-methoxyphenyl)pteridine  
40 2-amino-4-piperidino-6-(3,4-dimethoxyphenyl)pteridine  
30 41 2-amino-4-N-methylpiperazino-6-phenylpteridine  
42 2-amino-4-N-methylpiperazino-6-(4-chlorophenyl)-  
pteridine  
43 2-amino-4-N-methylpiperazino-6-(4-methoxyphenyl)-  
pteridine  
35 44 2-amino-4-methylpiperazino-6-(3,4-dimethoxy-  
phenyl)pteridine

45 2-amino-4-cyclopentylamino-6-(4-methoxyphenyl)-pteridine

46 2-amino-4-piperazino-6-phenylpteridine

5 47 2-amino-4-piperazino-6-(4-chlorophenyl)pteridine

48 2-amino-4-piperazino-6-(4-methoxyphenyl)pteridine

49 2-amino-4-piperazino-6-(3,4-dimethoxyphenyl)pteridine

50 2-amino-4-dibenzylamino-6-(3,4,5-trimethoxyphenyl)-pteridine

10 51 2-amino-4-morpholino-6-(3,4,5-trimethoxyphenyl)-pteridine

52 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)-pteridine

53 2-amino-4-adamantyl-6-naftylpteridine

15 54 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)-pteridine

55 2-amino-4-adamantyl-6-naftylpteridine

56 2-amino-4-morpholino-6-(3,4-formylidene-3,4-dihydroxyphenyl)pteridine

20 57 2-amino-4-dimethylamino-6-(3,4-formylidene-3,4-dihydroxyphenyl)pteridine

58 2-amino-4-cyclopentylamino-6-(3,4,dimethoxyphenyl)-pteridine

59 2-amino-4-dimethylamino-6-(3,4-dimethoxyphenyl)-pteridine

25 60 2-amino-4-dimethylamino-6-methylpteridine

61 2-amino-4-ethoxy-6-phenylpteridine

62 2-amino-4-propylamino-6-phenylpteridine

63 2-amino-4-propylamino-6-(3,4-dimethoxyphenyl)-pteridine

30 64 2-acetamido-4-hydroxy-6-(3,4-dimethoxyphenyl)-pteridine

65 2-acetamido-4-i-propoxy-6-(3,4-dimethoxyphenyl)-pteridine

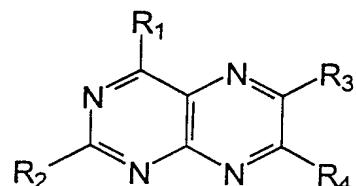
35 66 2-amino-4-ethoxy-6-(3,4-dimethoxyphenyl)pteridine

7. Pharmaceutical composition according to

claims 1-6, further comprising one or more other immunosuppressants chosen from the group comprising CyA, FK506, rapamycine (RPM), leflunomide, mofetil, MTX or 5-FU.

5

8. Compound having the general formula:



(I)

10 wherein:

R<sub>1</sub> and R<sub>2</sub> are independently amino, hydroxylamino, (mono- or di)C<sub>1-7</sub>alkylamino, (mono- or di)C<sub>1-7</sub>alkyloxyamino, (mono- or di)aryl amino, (mono- or di)C<sub>3-10</sub>cycloalkylamino, (mono- or di)hydroxyC<sub>1-7</sub>alkylamino, (mono- or di)C<sub>1-4</sub>alkyl-15 arylamino, mercaptoC<sub>1-7</sub>alkyl, C<sub>1-7</sub>alkyloxy or a saturated or unsaturated heterocyclic compound containing at least one nitrogen and optionally substituted by one or more C<sub>1-4</sub>alkyl, hydroxyC<sub>1-4</sub>alkyl, C<sub>1-4</sub>alkyloxy, halo, hydroxy, hydroxycarbonyl, C<sub>1-4</sub>alkyloxycarbonyl,

20 R<sub>3</sub> is an unsubstituted, monosubstituted or disubstituted aryl group (wherein the substituent may be, but not limited to, halogen, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl), aryl group bond to the pteridine ring via a saturated or unsaturated aliphatic spacer which may be halogenated or 25 hydroxylated, aliphatic substituent which may contain ether function, alcohol function, substituted or unsubstituted amino functions or C<sub>1-4</sub>alkyloxy,

R<sub>4</sub> is hydrogen, alkyl, alkoxy, substituted or unsubstituted aryl

30 or a pharmaceutical acceptable addition salt or a stereochemical isomeric form thereof.

9. Compound according to claim 8, wherein R<sub>1</sub> is

48

di(C<sub>1-4</sub>alkyl)amino, morpholinyl, piperidinyl, piperazinyl, C<sub>1-4</sub>alkylpiperazinyl, pyrrolidinyl or benzylamine.

10. Compound according to claim 8 or 9, wherein R<sub>2</sub> is ammonium, hydroxyammonium, (mono- or di)hydroxyl-C<sub>1-7</sub>alkylammonium.

5 11. Compound according to claims 8-10, wherein R<sub>3</sub> is benzyl, phenyl, styryl, phenyl(C<sub>1-4</sub>alkyloxy, phenyl-(C<sub>1-4</sub>alkyl) optionally substituted by one or more C<sub>1-4</sub>alkyl or halo.

10 12. Compound according to claims 8-11, wherein R<sub>4</sub> is hydrogen or C<sub>1-4</sub>alkyl.

13. Compound having the formula:

1 2-amino-4-pentoxy-6-styrylpteridine

2 2-amino-4-n-pentoxy-6-(1,2-dibromo-2-phenylethyl)-15 pteridine

3 2-amino-4-methoxy-6-styryl-7-methoxypteridine

6 2,4-diamino-6-phenyl-7-methylpteridine

14 2-amino-4-dimethylamino-6-phenylpteridine

15 2-amino-4-dimethylamino-6-(4-tolyl)pteridine

20 16 2-amino-4-dimethylamino-6-(4-methoxyphenyl)pteridine.

21 2-amino-4-diethylamino-6-phenylpteridine

22 2-amino-4-diethylamino-6-(4-chlorophenyl) pteridine

23 2-amino-4-diethylamino-6-(4-methoxyphenyl) pteridine

24 2-amino-4-diethylamino-6-(3,4-dimethoxyphenyl)-15 pteridine

25 2-amino-4-dibenzylamino-6-phenyl pteridine

26 2-amino-dibenzylamino-6-(4-chlorophenyl) pteridine

27 2-amino-4-dibenzylamino-6-(4-methoxyphenyl) pteridine

28 2-amino-4-dibenzylamino-6-(3,4-dimethoxyphenyl)-30 pteridine

29 2-amino-4-dipropylamino-6-phenylpteridine

30 2-amino-4-dipropylamino-6-(4-chlorophenyl)pteridine

31 2-amino-4-dipropylamino-6-(4-methoxyphenyl)pteridine

32 2-amino-4-dipropylamino-6-(3,4-dimethoxyphenyl)-35 pteridine

33 2-amino-4-morpholino-6-phenylpteridine

## 49

34 2-amino-4-morpholino-6-(4-chlorophenyl)pteridine  
35 2-amino-4-morpholino-6-(4-methoxyphenyl)pteridine  
36 2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)-  
pteridine  
5 37 2-amino-4-piperidino-6-phenylpteridine  
38 2-amino-4-piperidino-6-(4-chlorophenyl)pteridine  
39 2-amino-4-piperidino-6-(4-methoxyphenyl)pteridine  
40 2-amino-4-piperidino-6-(3,4-dimethoxyphenyl)-  
pteridine  
10 41 2-amino-4-N-methylpiperazino-6-phenylpteridine  
42 2-amino-4-N-methylpiperazino-6-(4-chlorophenyl)-  
pteridine  
43 2-amino-4-N-methylpiperazino-6-(4-methoxyphenyl)-  
pteridine  
15 44 2-amino-4-methylpiperazino-6-(3,4-dimethoxyphenyl)-  
pteridine  
45 2-amino-4-cyclopentylamino-6-(4-methoxyphenyl)-  
pteridine  
46 2-amino-4-piperazino-6-phenylpteridine  
20 47 2-amino-4-piperazino-6-(4-chlorophenyl)pteridine  
48 2-amino-4-piperazino-6-(4-methoxyphenyl)pteridine  
49 2-amino-4-piperazino-6-(3,4-dimethoxyphenyl)-  
pteridine  
50 2-amino-4-dibenzylamino-6-(3,4,5-trimethoxyphenyl)-  
pteridine  
25 51 2-amino-4-morpholino-6-(3,4,5-trimethoxyphenyl)-  
pteridine  
51 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)-  
pteridine  
30 53 2-amino-4-adamantyl-6-naftylpteridine  
54 2-amino-4-adamantyl-6-(3,4,5-trimethoxyphenyl)-  
pteridine  
55 2-amino-4-adamantyl-6-naftylpteridine  
56 2-amino-4-morpholino-6-(3,4-formylidene-3,4-  
35 dihydroxyphenyl)pteridine  
57 2-amino-4-dimethylamino-6-(3,4-formylidene-3,4-

50

dihydroxyphenyl)pteridine  
58 2-amino-4-cyclopentylamino-6-(3,4,dimethoxyphenyl)-  
pteridine  
58 2-amino-4-dimethylamino-6-(3,4-dimethoxyphenyl)-  
pteridine  
60 2-amino-4-dimethylamino-6-methylpteridine  
61 2-amino-4-ethoxy-6-phenylpteridine  
62 2-amino-4-propylamino-6-phenylpteridine  
63 2-amino-4-propylamino-6-(3,4-dimethoxyphenyl)-  
pteridine  
10 64 2-acetamido-4-hydroxy-6-(3,4-dimethoxyphenyl)-  
pteridine  
65 2-acetamido-4-i-propoxy-6-(3,4-dimethoxyphenyl)-  
pteridine  
15 66 2-amino-4-ethoxy-6-(3,4-dimethoxyphenyl)pteridine.

14. Use of a compound according to claims 8-13  
as a medicament.

15. Use of a compound according to claims 8-13,  
for the manufacture of a medicament for the treatment of  
20 autoimmuno disorders.

16. Use of a compound according to claims 8-13,  
for the manufacture of a medicament for the prevention of  
transplant-rejections.

17. Use of a compound according to claims 8-13,  
25 for the manufacture of a medicament for the treatment of  
inflammatory diseases.

18. Method for selecting immunosuppressive  
agents by a combination of at least three test systems  
based on MLR, aCD3 and aCD28.

## INTERNATIONAL SEARCH REPORT

Inte onal Application No  
PCT/EP 99/10320

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07D475/04 C07D475/08 A61K31/519 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 362 645 A (THOMAE GMBH DR K) 11 April 1990 (1990-04-11) claims	1-18
A	EP 0 290 819 A (BIORESEARCH SPA) 17 November 1988 (1988-11-17) claims	1-18
A	WO 95 31987 A (HOECHST AG ;PFLEIDERER WOLFGANG (DE); SCHMIDT HARALD (DE); HENNING) 30 November 1995 (1995-11-30) cited in the application claims	1-18

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the International search

23 March 2000

Date of mailing of the International search report

03/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentiaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Chouly, J

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 99/10320

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 32203 A (HOECHST AG ;PFLEIDERER WOLFGANG (DE); SCHMIDT HARALD (DE); HENNING) 30 November 1995 (1995-11-30) cited in the application claims	1-18
A	EP 0 108 890 A (WELLCOME FOUND) 23 May 1984 (1984-05-23) cited in the application claims	1-18
A	WO 98 04558 A (ARTEMIS INC ;ARAI TOSHIYUKI (JP); MAKINO KEISUKE (JP); MORI KENJIR) 5 February 1998 (1998-02-05) claims	1-18
A	WO 98 08516 A (ISHIHARA TAKAFUMI ;YOSHIKAWA JUNICHI (JP); OKAMURA MIKIO (JP); SUN) 5 March 1998 (1998-03-05) claims	1-18
P,A	EP 0 956 855 A (LEUVEN K U RES & DEV ;PFLEIDERER WOLFGANG (DE)) 17 November 1999 (1999-11-17) cited in the application claims	1-18
P,A	US 5 992 713 A (MANABAT GREGORIO S) 30 November 1999 (1999-11-30) the whole document	1-18

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Inte...inal Application No

PCT/EP 99/10320

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
EP 0362645	A 11-04-1990	DE 3833393 A	05-04-1990	AT 106728 T	15-06-1994
		AU 620645 B	20-02-1992	AU 4174989 A	05-04-1990
		DD 299062 A	26-03-1992	DE 58907824 D	14-07-1994
		DK 480489 A	02-04-1990	ES 2056174 T	01-10-1994
		FI 894615 A, B,	02-04-1990	HU 52504 A, B	28-07-1990
		IL 91814 A	27-02-1994	JP 2256676 A	17-10-1990
		MX 9203034 A	01-07-1992	NO 175979 B	03-10-1994
		NZ 230833 A	28-05-1991	PT 91836 A, B	30-04-1990
		SU 1720491 A	15-03-1992	ZA 8907414 A	26-06-1991
EP 0290819	A 17-11-1988	IT 1204612 B	10-03-1989	AT 97808 T	15-12-1993
		DE 3885933 D	13-01-1994	DE 3885933 T	14-04-1994
		DE 3885933 T	14-04-1994	ES 2061543 T	16-12-1994
		JP 2621925 B	18-06-1997	JP 63297326 A	05-12-1988
		JP 63297326 A	05-12-1988	US 5047405 A	10-09-1991
WO 9531987	A 30-11-1995	DE 4418096 A	30-11-1995	EP 0760664 A	12-03-1997
		JP 10504023 T	14-04-1998	US 5902810 A	11-05-1999
WO 9532203	A 30-11-1995	DE 4418097 A	30-11-1995	CA 2188267 A	30-11-1995
		CA 2188267 A	30-11-1995	EP 0760818 A	12-03-1997
		JP 10500417 T	13-01-1998	JP 10500417 T	13-01-1998
EP 0108890	A 23-05-1984	AU 572792 B	19-05-1988	AU 1925683 A	29-03-1984
		AU 1925683 A	29-03-1984	CA 1288099 A	27-08-1991
		CA 1288099 A	27-08-1991	DK 426083 A	21-03-1984
		DK 426083 A	21-03-1984	JP 1828391 C	15-03-1994
		JP 1828391 C	15-03-1994	JP 5033229 B	19-05-1993
		JP 5033229 B	19-05-1993	JP 59076086 A	28-04-1984
		JP 59076086 A	28-04-1984	US 4665182 A	12-05-1987
		US 4665182 A	12-05-1987	ZA 8306957 A	24-04-1985
		ZA 8306957 A	24-04-1985	DK 159184 A, B,	13-01-1985
		DK 159184 A, B,	13-01-1985	US 4701455 A	20-10-1987
WO 9804558	A 05-02-1998	AU 3707197 A	20-02-1998	CA 2233312 A	05-02-1998
		CA 2233312 A	05-02-1998	CN 1198161 A	04-11-1998
		CN 1198161 A	04-11-1998	EP 0884317 A	16-12-1998
WO 9808516	A 05-03-1998	EP 0908182 A	14-04-1999	JP 10338637 A	22-12-1998
		JP 10338637 A	22-12-1998		

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Inten. /inal Application No

**PCT/EP 99/10320**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
<b>EP 0956855</b>	<b>A</b>	<b>17-11-1999</b>	<b>NONE</b>
<b>US 5992713</b>	<b>A</b>	<b>30-11-1999</b>	<b>NONE</b>